Nanocurcumin Potential Effect of SOD Enzyme and Caspase-3 Expression in Lead-Acetate Induced Rats Ovarian Granulosa Cells

Anis Satus Syarifah[1], Sri Agus Sudjarwo[2], Hendy Hendarto[3], Reny I'tishom[4], Supriyanto[5]

[1]Assistant Professor, Nursing Professional Education Program, STIKES Pemkab Jombang, Indonesia, [2]Professor, Department of Pharmacology, Faculty of Veterinary Medicine, Universitas, Airlangga, Surabaya, Indonesia, [3]Professor, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas, Airlangga, Surabaya, Indonesia, [4]Associate Professor, Department of Medical Biology, Faculty of Medicine, Universitas, Airlangga, Surabaya, Indonesia, [5]Assistant Professor, Department of Nursing, Politehnik Kesehatan Kemenkes Surabaya, Indonesia

Abstract

Aim: This study investigated the potential effect of nanocurcumin on the increase of SOD enzyme expression and decrease of caspase-3 in lead acetate-induced rats ovarian granulosa cells.

Materials and Methods: Forty five female rats were divided into 5 groups, the negative control group (rats receiving corn oil, one hour later receiving distilled water), positive control group (rats receiving corn oil, one hour later receiving lead acetate of 40 mg/kg bw) and experimental groups 1, 2 and 3 (rats receiving nanocurcumin 50 mg, 100 mg and 200 mg/kg bw). One hour after administering the nanocurcumin, the rats received 40 mg/kg of lead acetate. All groups received oral treatment once a day for 26 days. On day 27 the rats were sacrificed and the expression of SOD and caspase-3 enzymes were measured using immunohistochemical methods.

Results: This study found that lead acetate decreased SOD enzyme expression and increased caspase-3. In contrast, nanocurcumin increased SOD enzyme and decreased caspase-3 expression in lead acetate-induced rats ovarian granulosa cells.

Conclusion: Nanocurcumin has potential as a strong natural antioxidant by affecting the increase of SOD and the decrease of caspase-3 cells in lead acetate-induced rats ovarian granulosa cells.

Keywords*: Antioxidants; lead acetate; SOD and Caspase-3; nanocurcumin*

Introduction

Lead (Pb) is one of the environmental pollutants that are dangerous, toxic, carcinogenic, biomagnificative, and bioaccumulative, as well as causing disorders and damages to reproductive system in animals and humans.[1] Continuous lead exposure will accumulate in the body, causing toxicity and potentially resulting in infertility,[2] reproductive system failure,[3] abortion, premature birth and fetal death.[4] Lead can also inhibit the work of enzymes, interfere with mineral absorption and easily bind to cysteine, lysine and histidine imidazole, and replace endogenous ions of the enzyme. The binding between Pb and protein will form a protein-metal binding, the metallotionin-Pb, and this binding will cause the enzyme to become inactive. Inhibition of this enzyme's action will affect physiological processes and can disrupt cell structure in the body's organs.[5]

Lead can affect SOD (superoxide dismutase) and caspase-3 enzymes. SOD is a metalloporfirin-protein that functions to convert *O2- to O2 and H2O2 and is an enzymatic metallo-enzyme antioxidant in the body. In its activity as an antioxidant, SOD depends on metal cofactors Cu, Zn, Mn and Fe. One of the highest activities of SOD is in the ovaries. Pb can bind to SOD

and replace endogenous ions from metallo-enzymes, forming Pb-metallotion which results in decreased SOD activity as an antioxidant.[6] Caspase-3 has an important role in cell apoptosis regulatory mechanisms.[7] The activity of caspase-3 is produced as an inactive enzyme (procaspase).[8] Pb exposure can cause an increase in caspase-3 through oxidative stress mechanisms. Lead causes an increase in ROS (Reactive Oxygen Species) followed by an increase in oxidative stress and Bax/ Bcl-2 ratio, as well as an increase in caspase-3 from the mitochondria.[9]

Curcumin functions as anti-bacterial, anti-inflammatory, chemopreventive, wound healer, anti-parasite and antioxidant.[10] Curcumin has antioxidant and ROS-reducing effects resulting from the action of its phenolic group (-OH).[11] Curcumin can reduce oxidative stress caused by lead toxicity[12] and can increase superoxide dismutase (SOD).[13] Curcumin is also able to inhibit the increase in caspase-3 by preventing the formation of hydroxyl radicals (OH*). The prevention of (OH*) formation by curcumin is by preventing Haber Weiss reaction and Fenton reaction.[14]

Materials and Methods

Chemicals

Lead acetate (PbAc) was purchased from Sigma-Aldrich.co. USA, Linear formula: Pb (CH3CO2) 2.3H2O), (Product No: CAS 6080-56-4, molecular weight (MW): 379.33 g/mol and Curcumin Product No: CAS 458-37-7 molecular weight (MW): 368.38 g/mol, product of China. (Curcuma longa (turmeric) powder, > 65%.

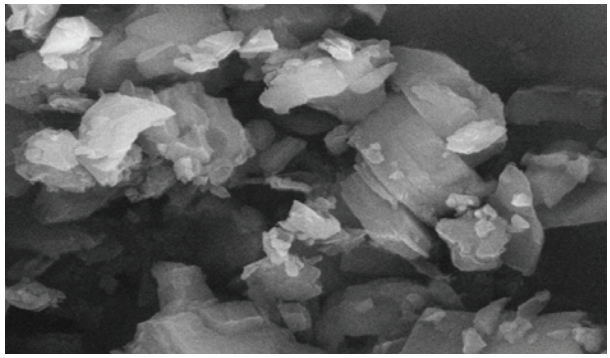
Making process of nanocurcumin

Nanocurcumin was made using curcumin powder from the rhizome of Curcuma longa (turmeric) using the miling method. Curcumin was milled by pounding by cubic zirconia balls, with a ratio of curcumin : cubic zirconia balls = 1: 10. The curcumin and cubic zirconia balls were put into a tube and milled on a High Energy Miling (HEM) machine. The setting time of milling was 5 minutes milling, 5 minutes rest until the effective time of milling, which was 20 minutes outside break time, was reached. Nanocurcumin was made at the Physics Laboratory, Airlangga University, Surabaya, Indonesia.

Analysis of nanocurcumin characteristics

After milling, the morphology of curcumin showed a more regular crystal shape with an average diameter of less than 200 nm. The morphology of curcumin prior to milling appeared as irregular plates with mean diameter of more than 1000 nm. The characteristic analysis of nanocurcumin size was carried out using Scanning Electron Microscopy (SEM) at Robotics Laboratory, ITS, Surabaya, Indonesia. The differences in the characteristics of curcumin before and after millling are shown in Figure 1.

(A)



(B)

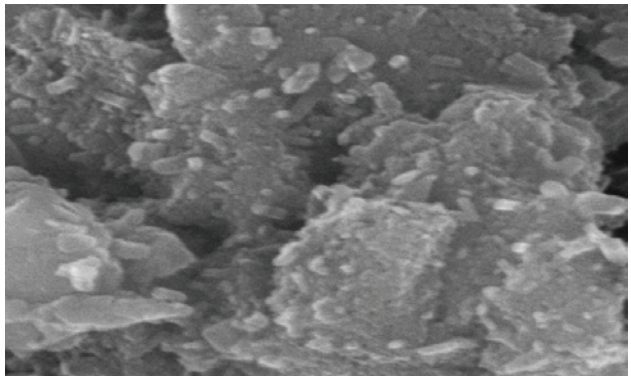


Figure 1. Curcumin before and after milling. (A) Curcumin before milling with a size > 1000 nm; (B) Curcumin after milling with a size of < 200 nm.

Making of nanocurcumin solution

Corn oil is the best solvent for nanocurcumin compared to butter, milk and water,[15] so this study used corn oil as a solvent. A solution was made by dissolving 2 grams of nanocurcumin with corn oil to 200 ml, so that 1 ml of the solution contained 10 mg of nanocurcumin.

Experimental animals

This study had passed the ethical test of the Ethics

Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, and obtained ethical eligibility No: 2.KE.170.08.2019 dated 29 August 2019. The experimental animals were female wistar rats weighing around 180-200 g, aged 2.5 - 3 months, obtained from the Bandung Institute of Technology (ITB), Bandung, Indonesia. The rats were placed in cages in an air-conditioned room with temperatures maintained at 26C ° - 2C ° and 12 hours in a light and dark cycle.

Experimental design

This study used 45 female rats which were divided into 5 groups, the negative control group (C-), where the rats received corn oil and one hour later received distilled water; positive control group (C +) where the rats received corn oil and one hour later received lead acetate of 40 mg/kg bw/day; experimental group 1 (E1), where the rats received nanocurcumin of 50 mg/kg bw/day; experimental group 2 (E2), where the rats received nanocurcumin of 100 mg/kg bw/day; and experimental group 3 (E3) where the rats received nanocurcumin 200 mg/kg body weight/day. One hour after giving nanocurcumin, experimental groups 1,2 and 3 were given with lead acetate of 40 mg/kg bw/day. The treatments were carried out every day at 09.00 AM for 26 days. On day 27 the rats were sacrificed, the ovaries were cleaned of connective tissue, washed with 0.9% physiological NaCl and implanted in paraffin blocks. Furthermore, the examination of SOD and caspase-3 enzyme expression in the granulosa cell was carried out using immunohistochemical methods.

Immunohistochemical examination

Subsequently, serial paraffin blocks, in which the ovary had been already implanted, were incised to a thickness of 4 - 6 □m. Representative incisions were selected for immunostaining procedure. The tissue incisions were stained with streptavidin method using immunoperoxidase. The next step was calculating the expression of the enzymes SOD and caspase-3 in 400x magnification. The presence of SOD and caspase-3 enzymes was indicated by the intensity of dark brown color. Observations were made quantitatively by counting the number of positive cells in each visual field, counting up to 10 visual fields. The number of positive cells for each visual field was summarized and divided by 10, and the final result was the mean number of positive cells per visual field.

Statistical Analysis

Data are presented as mean ± standard deviation. The comparative test used was Kruskall-Wallis Test to determine the differences between groups, followed by Mann-Whitney test to determine differences between the groups.

Results

Potential effect of nanocurcumin on the increase of SOD enzyme expression in lead acetate-induced rats ovarian granulosa cells

The results of Kruskal-Wallis test showed differences in the expression of the enzyme SOD (Kruskal-Wallis H = 23.625; df = 4; p = .000). Furthermore, to determine the differences between groups, analysis using Mann-Whitney Test was carried out, as in Table 1.

Tabel 1: Potential effect of nanocurcumin on SOD enzyme expression in lead acetate-induced rats ovarian granulosa cells (mean ± standard deviation)

Groups	n	SOD expression (%/micro)	Minimum	Maximum
Negative control	9	1.6 ± 1,2 a	0	3.5
Positive control	8	0.4 ± 0,3 b	0	1.2
Experimental 1	9	5.1 ± 3,5 c	0.6	12.4
Experimental 2	9	8.2 ± 10,5 c	0.3	28.8
Experimental 3	8	15.0 ± 12,6 c	1.0	35.5

[a, b,c] Different superscript within each column indicates significant difference between the means ($p < .05$).

Table 1 shows that the highest mean of SOD enzyme expression in rats ovarian granulosa cells is in experimental group 3 (x = 15.0; sd = 12.6%/micro), and the lowest is in positive control group (x = 0.4; sd = 0.3%/micro). Negative control group was different from positive control group (p = 0.017), experimental group 1 (p = 0.010), experimental group 2 (p = 0.038), and experimental group 3 (p = 0.005). Positive control group was different from experimental group 1 (p = 0.001), experimental group 2 (p = 0.001) and experimental group 3 (p = 0.001). Experimental group 1 was similar to experimental group 2 (p = 0.627) and experimental group 3 (p = 0.102). Experimental 2 group was similar to experimental group 3 (p = 0.178).

These results indicated that the administration of nanocurcumin increased the expression of SOD enzyme in lead acetate-induced rats ovarian granulosa cells starting from doses of 50, 100 and 200 mg/kg bw. This difference can be seen in Figure 2.

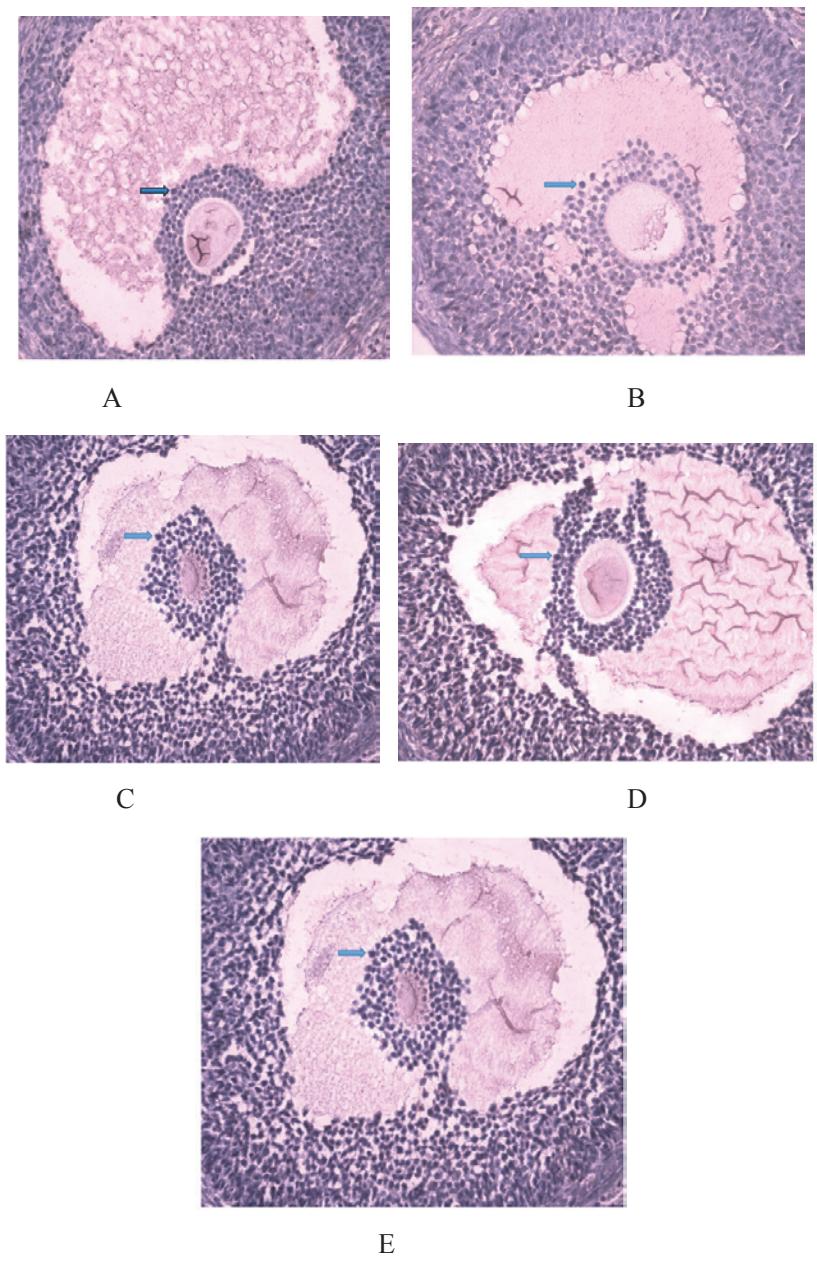


Figure 2. Expression of SOD in rats ovarian granulosa cells (A) K- group; (B) K+ group; (C) E1 group; (D) E2 group; (E) E3 group. Observation used light microscope with a magnification of 400x

Potential effect of nanocurcumin on decrease in caspase-3 expression in lead acetate-induced rats ovarian granulosa cells

The Kruskal-Wallis test showed differences between groups in the expression of caspase-3 (Kruskal-Wallis H = 27.321; df = 4; p = .000). Furthermore, to identify differences between groups, analysis using Mann-Whitney Test was carried out, as shown in Table 2.

Table 2. Potential effect of nanocurcumin on the expression of caspase-3 in lead acetate-induced rats ovarian granulosa cells (mean ± standard deviation)

Groups	n	Caspase-3 expression (%/micro)	Minimum	Maximum
Negative control	9	3.3 ± 5.9 a	0.1	17.3
Positive control	8	45.1 ± 42.2 b	2.5	103.6
Experimental 1	9	2.0 ± 1.1 a,d	0.6	3.6
Experimental 2	9	1.0 ± 1.5 a,c	0	4.8
Experimental 3	8	0.1 ± 0.1 c	0	0.3

[a, b, c, d] Different superscript within each column indicates significant difference between the means ($p < .05$).

Table 2 shows the highest mean is in the positive control group (45.1 ± 42.2%/micro) and the lowest in the experimental group 3 (0.1 ± 0.1%/micro). Negative control group was different from positive control group (p = .003), similar to experimental group 1 (p = .169) and experimental group 2 (p = .449), but different from experimental group 3 (p = .003)). Positive control group was different from experimental group 1 (p = .001), experimental group 2 (p = .001) and experimental group 3 (p = .001). Experimental group 1 was different from experimental group 2 (p = .042) and different from experimental group 3 (p = .000). Experimental group 2 was similar to experimental group 3 (p = .059). This proves that nanocurcumin starting at doses of 50, 100 and 200 mg/kg bw can reduce caspase-3 in lead acetate-induced rats ovarian granulosa cells. This difference can be seen in Figure 3.

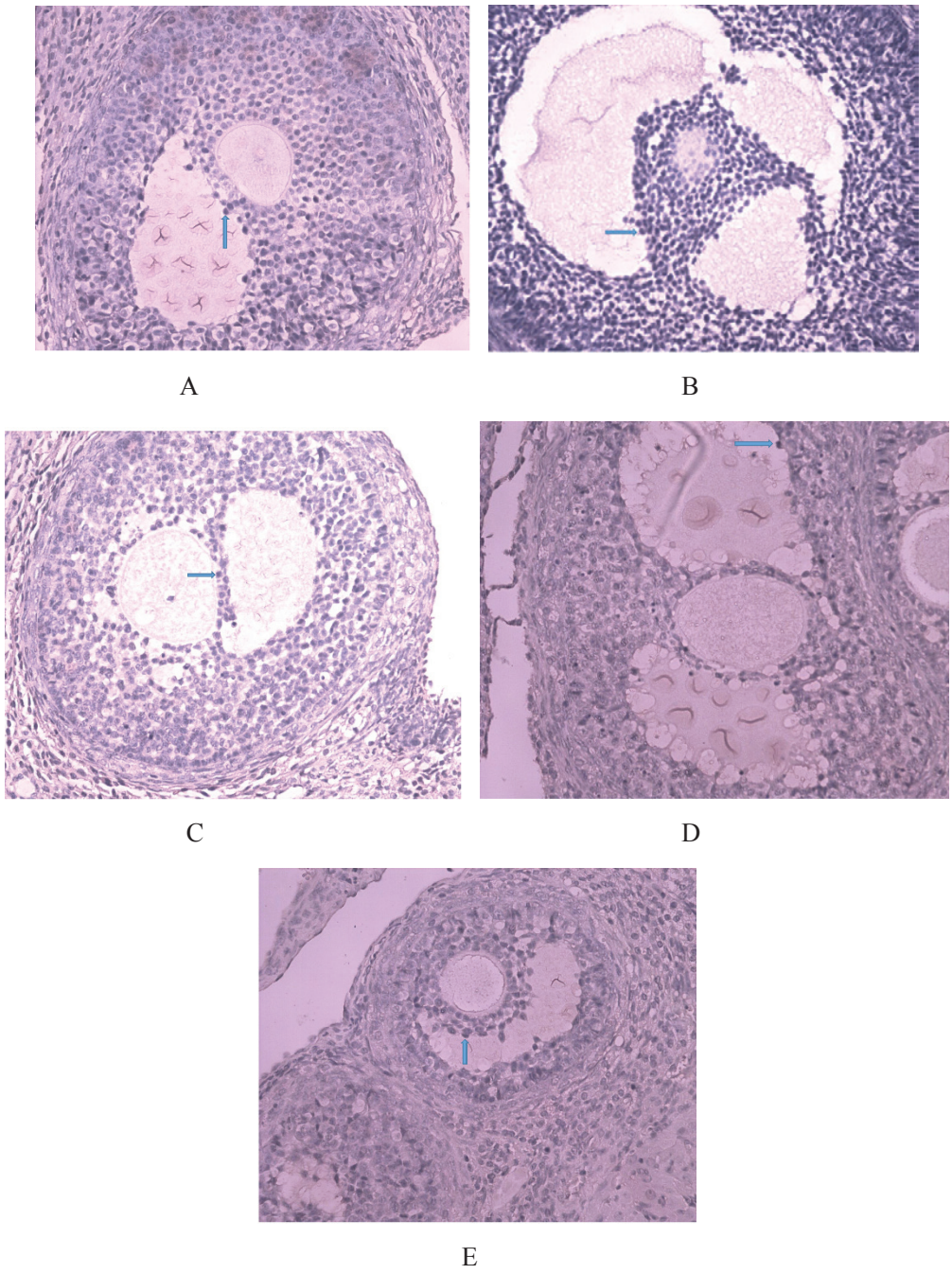


Figure 3. Expression of caspase-3 granulosa cells in rats ovaries with 400x magnification. (A) K- group; (B) K+ group; (C) E1 group; (D) E2 group; (E) E3 group. Observation used light microscope with a magnification of 400x. Arrow ( ) indicates an example of caspase-3 expression.

Discussion

This study proved that exposure to lead acetate as much as 40 mg/kg bw in rats decreased the expression of enzyme SOD in ovarian granulosa cells. We found that mean SOD enzyme expression in control group (K+) was lower than that in control group (K-). This finding was in line with the previous research that the administration of lead acetate in a dose of 24 mg/kg bw/day per sonde for one month resulted in lower blood erythrocyte SOD enzyme levels compared to control group that received water,[16] also stated that exposure to lead acetate of 100 mg/kg bw mixed with corn oil in rats decreased hepatic

SOD enzymes compared to that of control group.[17] Other researchers also reported that white rats injected with lead acetate of 20 mg/kg bw for 11 days had lower mean testicular SOD enzymes than control group.[18]

Lead acetate within cells is degraded, releasing Pb^{2+}.[19] Pb^{2+} can inhibit the action of SOD enzyme, which is a metalloenzyme, and requires metal cofactors for its antioxidant activity. SOD binds metal ions of zinc (Zn) and copper (Cu) that act as the cofactor.[20] Pb^{2+} works by binding to proteins and replacing endogenous ions (Cu/Zn) of SOD enzyme, so that this enzyme is not active as an antioxidant.[21]

In this study, the administration of nanocurcumin was shown to increase the expression of the enzyme SOD. The higher the nanocurcumin dose, the higher the expression of SOD enzyme in ovarian granulosa cells of rats exposed to lead acetate in a dose of 40 mg/kg bw. This can be observed from the mean SOD enzyme expression in E3 rats which was higher than that in E2 group and mean SOD in E2 group which was higher than that in E1 group. This finding was in line with the previous research who found that the administration of curcumin of 200 mg/kg bw/day orally and Pb of 50 mg/kg bw intraperitoneally for 7 days caused SOD expression in the liver of white rats to be higher than that in control group receiving Pb in a dose of 50 mg/kg bw intraperitoneally for 7 days.[22] Another study found that the provision of lead acetate of 100 mg/kg bw/day to white rats orally for 4 weeks, followed by curcumin of 400 mg/kg bw/day diluted with corn oil, caused a higher hepatic SOD expression than in control group.[23]

Curcumin has antioxidant and ROS-reducing effects due to its phenolic group (-OH).[24] The activity of free radical scavenging by curcumin is influenced by its phenolic hydroxyl groups. The energy of the curcumin phenolic OH group is lower (5.04 kcal mol) compared to the dissociation energy of the C-H bond in beta-dicetone curcumin group, so that the tendency of the antioxidant mechanism of curcumin is on the H atom in the phenolic group.[25] The antioxidant activity of nanocurcumin in increasing the expression of the SOD enzyme in rats granulosa cells exposed to lead acetate 40 mg/kg bw was by inhibiting the formation of superoxide radicals (O_2^-*)[26] through the inhibition of oxidative phosphorylation by suppressing the activity of cytochrome c oxidative enzymes.[27] Inhibition of oxidative phosphorylation by cytochrome P450 will reduce superoxide free radicals and increase the enzyme superoxidant dismutase (SOD).[26]

The results of this study also showed that oral administration of lead acetate (Pb) of 40 mg/kg bw/day increased the expression of caspase-3 enzyme in rats ovarian granulosa cells compared to that of negative control group (K-) which received distilled water. This finding was in line with a previous study,[28] who found an increase in testicular caspase-3 expression of white rats receiving lead acetate (Pb) of 30 mg/kg bw/day per sonde for 60 days compared to that of the control group. In vitro studies by He *et al.*,(2016) [29] showed that lead acetate in doses of 0.5; 1.0; 2.0 mol/l in normal rats renal epithelial cells for 12 h led to an increase in caspase-3 and caspase-9. Another study concerning intra-peritoneal injection of lead acetate as much as 20 mg/kg bw for 11 days in white rats resulted in increased caspase-3 testicular tissue compared to that of control group.[18].

Pb exposure can cause an increase in caspase-3 expression through oxidative stress mechanisms. The results showed that lead acetate caused an increase in ROS followed by an increase in oxidative stress. The presence of oxidative stress triggers an increase in the permeability of mitochondrial membrane which triggers the release of Smac/DIABLO and activation of pro-apoptotic protein (Bax/Bcl-2). Increasing Bax/Bcl-2 ratio increases the release of chytochrome c from mitochondria to cytosol.[9] In the cytosol, cytochrome-c binds to Apaf-1 (apoptotic protease activating factor-1) using energy from ATP and activates pro-caspase-9 in the form of an apoptosome. This apoptosome is what makes caspase-9 active. Active Caspase-9 will activate caspase-3.[30]

The results of this study also proved that nanocurcumin had the effect of reducing caspase 3. Nanocurcumin acts in the reduction of caspase- by inhibiting the formation of hydroxyl radicals (OH*) which are formed due to the encounter of superoxide ions (O2*-) with hydroperoxide (H2O2). This reaction requires the transition metal Fe^{++} (Cu^{+}) which is called the Haber Weiss reaction. Hydroxyl radicals (OH*) are also formed from H2O2 that reacts with the transition

metals Fe^{++} and Cu^{+}, which is called the Fenton reaction. To prevent Haber Weiss reaction and Fenton reaction, the presence of Fe++ and C+ ions must be prevented by binding Fe^{++} and Cu^{+}.[31] The inhibition of OH* formation results in increased mitochondrial phosphorylation, thus inhibiting the expression of Bcl-2 associated killer (Bak) and Bcl-2 associated death molecule (Bad) as pro-apoptotic proteins known as Bax subfamily.[32] Inhibition of Bak and Bad expression leads to an increase in anti-apoptotic proteins Bcl-2 and Bcl-xL. The increase in Bcl-2 and Bcl-xL inhibits the release of cytochrome c (cyt c) so that the binding of cytochrome c, apaf-1 and caspase-9 is minimal and the activation of procaspase-3 by caspase-9 does not occur, which will result in decreased expression of caspase-3.[33]

Conclusion

Nanocurcumin has the potential as an antioxidant to increase the expression of the first-line SOD enzyme and reduce caspase-3 in lead acetate-induced rat ovarian granulosa cells.

Conflict of Interest: There is no conflict of interest in this article.

Ethical Clearance: Taken from Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia

Souce of Funding : None

References

1. Kabeer, A., Muhammad Mailafiya, M., Danmaigoro, A., Abdul Rahim, E., & Bu Bakar, M. Z. A. (2019). Therapeutic potential of curcumin against lead-induced toxicity: A review. Biomedical Research and Therapy, 2019;*6*(3):3053–66.
2. Singh, N., Kumar, A., Gupta, V. K., & Sharma, B. Biochemical and Molecular Bases of Lead-Induced Toxicity in Mammalian Systems and Possible Mitigations. Chemical Research in Toxicology, 2018;*31*(10):1009–21.
3. Assi, M. A., Noor, M., Hezmee, M., Haron, A. W., Yusof, M., & Sabri, M. The detrimental effects of lead on human and animal health. Veterinary World,2016; *9*: 660–71.
4. Dumitrescu, E., Chiurciu, V., Muselin, F., Popescu, R., Brezovan, D., & Cristina, R. T. Effects of long-term exposure of female rats to low levels of lead: Ovary and uterus histological architecture changes. Turkish Journal of Biology,2015;*39*(2):284–9.
5. Suryatini, K. Y., & Rai, I. G. A. (2018). Lead (Pb) and Its Effect of Reproductive System. Emasains : Jurnal Edukasi Matematika Dan Sains (Emasains: Journal of Mathematics and Sciende Education),2018;*VII*(1):1–6.
6. AL-Megrin, W. A., Soliman, D., Kassab, R. B., Metwally, D. M., Dina, D. M., & El-Khadragy, M. F. Coenzyme Q10 Activates the Antioxidant Machinery and Inhibits the Inflammatory and Apoptotic Cascades Against Lead Acetate-Induced Renal Injury in Rats. Frontiers in Physiology,2020;*11*(2):1–13.
7. Anandani, E. tantri, Kusnanto, P., & Purwanto, B. Apoptosis in Colon Cancer Cells *(Cell Line)*,2017; *9*: 23–30.
8. Banu, M. A. F., & Abhilasha, R. Patterns of Apoptosis – A Review. International Journal of Pharmaceutical Sciences Review and Research,2018; 50(2)(01): 1–6.
9. Thangarajan, S., Vwdagiri, A., Somasundaram, S., Sakthimanogaran, R., & Murugesan, M. Neuroprotective effect of morin on lead acetate-induced apoptosis by preventing cytochrome c translocation via regulationof Bax/Bcl-2 ratio. Neurotoxicology and Teratology,2018; 66 (9): 35-45.
10. Bodhankar, M. M., & Chikhle, S. Various Approaches Towards Enhancement of Bioavailability of Curcumin-a Potent Phytochemical. World Journal of Pharmaceutical Research,2019; *8*(1): 606–26.
11. Ansar, S., Farhat, S., Albati, A. A., Abudawood, M., & Hamed, S. Effect of curcumin and curcumin nanoparticles against lead induced nephrotoxicity. Biomedical Research, 2019;30(1): 57–60.
12. Abu-Taweel, G. M. Curcumin attenuates lead (Pb)–induced neurobehaviorl and neurobiochemical dysfunction : A Review. International Journal of Pharmacy and Pharmaceutical Sciences,2018;*10*(8):23–8.
13. Azami S.H., Hamid Nazarian H., Mohammad-Amin Abdollahifar M. A., Farsani M.A., Banihosseini S. Z. Marefat Ghaffari Novin M.G. Curcumin delays oocyte apoptosis through overexpression of *BCL-2* gene in young and middle-aged mouse models. International Journal of Women's Health and Reproduction Sciences,2020; 8 (1): 53-60.

14. Wang, S., He, G., Chen, M., Zuo, T., Xu, W., & Liu, X. The Role of Antioxidant Enzymes in the Ovaries. Oxidative Medicine and Cellular Longevity, 2017: 1–14.
15. Shishu, Maheswari, M., Comparative Bioavaibility of curcumin, turmeric, and biocurcumax, in tradisional vehicles using non-everted rat intestinal sac model. J of Fuctional Food,2010; 2 : 60-5
16. El-Sherbini, E. S., El-Sayed, G., El Shotory, R., Gheith, N., Abou-Alsoud, M., Harakeh, S. M., & Karrouf, G. I. Ameliorative effects of L-carnitine on rats raised on a diet supplemented with lead acetate. Saudi Journal of Biological Sciences, 2017;24(6): 1410–7.
17. Abdelhamid, F. M., Mahgoub, H. A., & ateya, A.I. Ameliorative effect of curcumin against lead acetat – induced hemato-biochemical alteration, hepatotoxicity and testicular oxidative damage in rats. Environmental Science and Pollution Research,2020:1-16.
18. Sudjarwo, S. A., Anwar, C., Wardani, G., Eraiko, K., & Koerniasari. Antioxidant and anti-caspase 3 effect of chitosan-Pinus merkusii extract nanoparticle against lead acetate-induced testicular toxicity in rat. Asian Pacific Journal of Reproduction,2019;*8*(1):13–9.
19. Albarakati, A. J. A., Baty, R. S., Aljoudi, A. M., Habotta, O. A., Elmahallawy, E. K., Kassab, R. B., & Abdel Moneim, A. E. Luteolin protects against lead acetate-induced nephrotoxicity through antioxidant, anti-inflammatory, anti-apoptotic, and Nrf2/HO-1 signaling pathways. Molecular Biology Reports,2020;1(3):1-13.
20. Ighodaro, O. M., & Akinloye, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine,2018;*54*(4): 287–93.
21. Zhai, Q., Narbad, A., & Chen, W. Dietary strategies for the treatment of cadmium and lead toxicity, Nutrients,2015; 7(1): 552-72.
22. Alhusaini, A., Fadda, L., Hasan, I. H., Zakaria, E., Alenazi, A. M., & Mahmoud, A. M. Curcumin ameliorates lead-induced hepatotoxicity by suppressing oxidative stress and inflammation, and modulating akt/gsk-3β signaling pathway. Biomolecules,2019; 9(11):1-17.
23. Suryatini KY, Rai IGA. Logam Berat Timbal (Pb) dan Efeknya pada Sistem Reproduksi. Emasains J Edukasi Mat dan Sains. 2018;VII(1):1–6.
24. HE XY, YUAN LY, LI YT, LI M, CHEN Y, YUAN H, et al. Cytotoxic Responses and Apoptosis in Rat Kidney Epithelial Cells Exposed to Lead. Biomed Environ Sci. 2016;29(7):529–33.